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S/N 10/594100 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Geng et al.	Examiner:	Maier, Leigh C.
Serial No.:	10/594100	Group Art Unit:	1623
Filed:	June 29, 2007	Docket No.:	09458.1045USWO

Title: ALGIN OLIGOSACCHARIDES AND THE DERIVATIVES THEREOF AS WELL AS THE MANUFACTURE AND THE USE OF THE SAME

DECLARATION UNDER 37 CFR §1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, Meiyu Geng, pH.D, hereby declare as follows:

1. I obtained my bachelor degree in Medicine in 1986 and master degree in pharmacology in 1989 from Shandong Medical University (Now Shandong University). I got my Ph.D in Pharmacy from Tokyo University in 1996.

2. I have worked in Marine Drug and Food Institute of Ocean University of China since 1989. I have started from the discovery to the development of carbohydrate-based drugs covering a descriptive phenomenon into molecular understanding in Alzheimer's treatment to cancer therapy in the past decade. Major interests are mainly focused on the research and development of targeted molecular agents in particular Aβ-targeting inhibitors, and deciphering of the possible molecular mechanisms in signal transduction. Currently, I am also focusing on characterizing genomics-based new targets and investigating the impact of biomarkers in AD progression and therapy response as well.

Based on the established glyco-microarray technique for high-throughput and micro-scale screening of biologically active marine-derived oligosaccharides, I have found a series of potential oligosaccharide-based drug candidates, including anti-AD drug candidate oligomannurarate and heparanase inhibitor JG3. During the past decades, more than 60 papers have been published in peer-reviewed journals and over 10 patents have been filed and 5 patents have been authorized.

I found that oligomannurarate, a novel marine-derived oligosaccharide, inhibits the entire fibril-forming process by stabilizing Aβ in an α-helical state, by driving disassembled fibrils into non-toxic conformers both in vitro and in a transgenic mouse model. Notably, this efficacy occurs via the binding capacity of oligomannurarate for N-terminus and β-hairpin species at different stages by simultaneously targeting SNK and HHQK domains on Aβ peptide. These features, together with good oral bioavailability, blood-brain barrier

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accessibility, and favorable safety and tolerability in newly completed Phase I clinical trials, make oligomannurarate both a prophylactic and therapeutic drug candidate for AD therapy. Now, oligomannurarate is under phase II clinical trial in China.

Inhibitors of tumor angiogenesis and metastasis are increasingly emerging as promising agents for cancer therapy. Recently, heparanase inhibitors have offered a new avenue for such work because heparanase is thought to be critically involved in the metastatic and angiogenic potentials of tumor cells. I found that oligomannurarate sulfate (JG3), a novel marine-derived oligosaccharide, acts as a heparanase inhibitor to inhibit tumor angiogenesis and metastasis both in vitro and in vivo by combating heparanase activity via binding to the KKDC and QPLK domains of the heparanase molecule, making JG3 a promising candidate agent for cancer therapy.

In addition, I have also discovered another sulfated polymannuroguluronate (SPMG), extracted from brown algae followed by chemical modification, inhibited HIV replication via its binding to the V3 region of the capsid glycoprotein molecule of the virus, gp120, therefore interrupting the binding of V3 region to CXCR4 and CCR5 (both are the co-receptors to CD4 molecule) and further preventing the entry of HIV into the host cells.

3. I am one of the inventors for the invention described in US Patent Application No. 10/594100 and am familiar with the subject matter thereof.

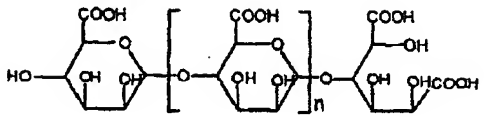
Alzheimer's disease (AD) is a devastating neurological disorder that affects more than 37 million people worldwide. The economic burden of AD is massive. Currently approved drugs for AD ameliorate symptoms for a short time by boosting levels of neurotransmitters, but do not alter the general progression or outcome of the disease.

Intense efforts have been devoted to finding disease-modifying therapies that target the underlying AD pathogenic molecules. Of these, β-amyloid peptide (Aβ), a 39-43 residue cleavage product of amyloid precursor protein, is the main component of senile plaques of AD, constitutes the focus of current interest.

Since amyloid fibril formation is a multi-stage process involving different Aβ species at different stages, an exciting current anti-AD strategy is to challenge mechanism-based multi-targeting agents. To this end, inhibitors should be broadly active across multiple stages of fibrillation. Such ideal agents are thus anticipated to stabilize Aβ in monomeric state that are unable to further assemble, favor the disassembly of high molecular-weight oligomers and fibrillar deposits in non-toxic conformers, and encourage its clearance through normal pathways by maintaining the Aβ in a monomer state.

With the availability of various synthetic Aβ species and a marine-derived carbohydrate library in our lab, a comprehensive screening program was undertaken. Oligomannurarate, an acidic oligosaccharide obtained from degradation and subsequent chemical modification, stood out as a full inhibitor of β-amyloid cascades by binding to Ser26-to-Lys28 (SNK) residues and simultaneously to the HHQK motif in Aβ peptide. oligomannurarate arrests fibril formation by stabilizing Aβ in an α-helix, and destabilizes fibrils into non-toxic conformers both *in vitro* and in a transgenic mouse model. The applied patent with No. 10/594100 generated from this product oligomannurarate.

4. Under my direction, the following comparative experiments were conducted for the purposes of showing that a different structure is obtained in 2002/0016453 than that of the following structure:



as recited in the claims in US Patent Application No. 10/594100.

Experiment 1

150 g polymannuronic acid (provided by Lantai Pharmacy, Qingdao, China) were dissolved in distilled water with 28 g lithium hydroxide monohydrate (provided by Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) in a final volume of 600 ml. 100 ml of 30% hydrogen peroxide (provided by Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) and 0.65 g ferrous sulfate heptahydrate (provided by Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) were added to the solution. And the mixture was reacted for 4 h. Then 50 ml 30% hydrogen peroxide were added to react for another 2 h. The mixture was then heated to 60 ℃ for 30min. The react were stopped by addition of sufficient Na_2S2O_3 (provided by Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). After cooling to room temperature, the pH was adjusted to 7.0-7.2 with 4 M NaOH. 4 times of 95% ethanol was added gradually with stirring. The solid was collected by vacuum filtration, and washed with 95% ethanol at least three times. Finally, the solid was dried under vacuum. The yield of product was 43 g.

The product was analyzed in a Bio-gel P-4 column (Extrafine, 1×200 cm). About 50mg degraded polymannuronic acid in 200μl water was applied to the column and eluted with 0.5 M NH_4HCO_3 at a flow rate of 2 ml/h. Fractions of 1 ml were collected and 50 μl was taken for hexuronic acid analysis with carbazole reagent method. The OD values at 530 nm to the effluent volume curve were shown as figure 1. Each peak was collected and dried under vacuum. The peaks were analyzed by MS and confirmed to be 1 to 11 mers of oligomannuronic acid.

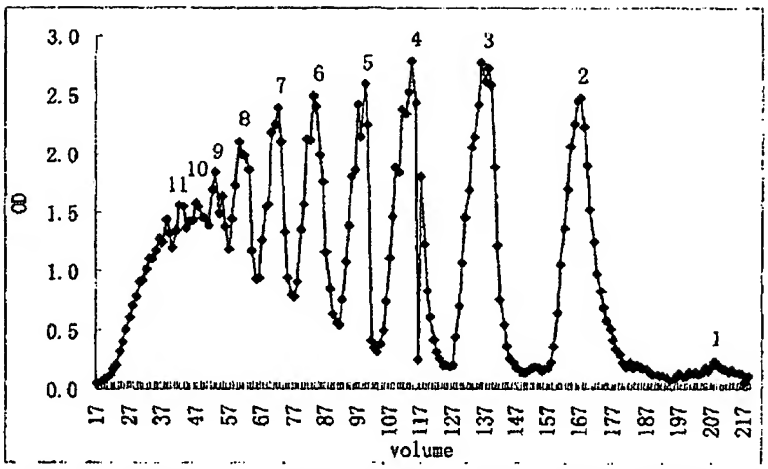
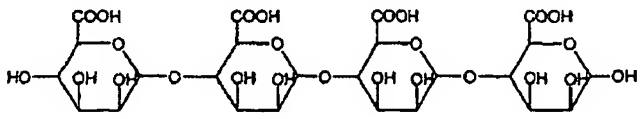


Fig. 1. Separation of polymannuronate-derived oligosaccharides by Bio-Gel P-4 chromatography. Mannuronate oligosaccharides degraded from polymannuronate were size-separated by application to a Bio-Gel P-4 (Extrafine) column (1× 200 cm) at a flow rate of 2 ml/h in 0.5 M NH_4HCO_3. Fractions (1 ml) were collected and analyzed using the carbazole reagent method.

Here we take 4-mer as an example to show the characterization of its structure.

1. Mass Spectrum

MS analysis of 4-mer was performed using Quattro Micro mass spectrometer (Waters) equipped with a MS pump and an autosampler. The mass spectrometer was set to full MS scan under negative ion mode. The results showed that the $[M-H]^-$ is 721.3, indicating that its molecular weight is 722, which matched to the following structure:



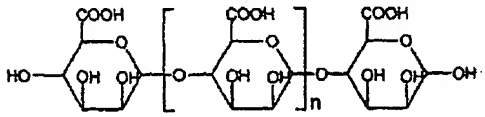
2. NMR

The NMR analysis of 4-mer was performed with a JNM-ECP600 spectrometer (JEOL, Japan). The chemical shift of H-1 at the reducing end was found at 5.21 ppm (H-1r α) and 4.91

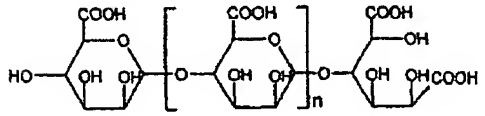
ppm (H-1r β), indicating the existence of the reducing end. The chemical shift of H-1, H-2, H-3, H-4, H-5 at the middle sugar residues were 4.69, 4.03, 3.75, 3.93 and 3.69 ppm, matched very well to the reference patent (4.67, 4.02, 3.73, 3.89 and 3.69 ppm), indicating the same chemical structure. The detected ^{13}C-NMR spectrum of 4-mer revealed that the chemical shift of C-1 at the reducing end is 94 ppm (C-1rα at 93.54 and C-1rβ at 93.74 ppm), also suggesting the existence of the reducing end (the reported chemical shift of C-1r is about 94 ppm). The chemical shift of C-1 at the middle and non-reducing end sugar residues were 99.08 and 100.15 ppm, matched very well to that of the reported (about 101 ppm).

The UV, IR and CD spectrum of 4-mer were also analyzed. All the data strongly suggested that the chemical structure of the product is composed of β-D-mannuronic acids linked by 1,4 glycosidic bonds (structure A), quite different to the structure in the claims in US Patent Application No. 10/594100 (structure B).

A



B



I declare under the penalty of perjury of the laws of the United States of America that the foregoing is true and correct to the best of my information and belief.

Signed by Gengmeiyu Print name GENG MEI YU

Date 2009.7.20 Place Qingdao, China